DESCRIPTION

METHOD OF PURIFYING REDUCED COENZYME Q_{10}

TECHNICAL FIELD

The present invention relates to a method of purifying reduced coenzyme Q_{10}. Reduced coenzyme Q_{10} shows higher level of oral absorbability as compared with oxidized coenzyme Q_{10} and is a compound useful as an ingredient in good foods, functional nutritive foods, specific health foods, nutritional supplements, nutrients, animal drugs, drinks, feeds, cosmetics, medicines, remedies, preventive drugs, etc.

BACKGROUND ART

It is known that reduced coenzyme Q_{10} can be prepared by producing coenzyme Q_{10} in the conventional manner, for example by synthesis, fermentation, or extraction from natural products, and concentrating a reduced coenzyme Q_{10}-containing eluate fraction resulting from chromatography (c.f. JP-A-10-109933). It is also described in the above-cited publication that, in this case, the chromatographic concentration may be carried out after reduction of oxidized coenzyme Q_{10}, occurred as an impurity in the reduced coenzyme Q_{10}, with a reducing agent such as sodium borohydride or sodium dithionite (sodium hyposulfite), or reduced coenzyme Q_{10} may be prepared by reacting the reducing agent mentioned above with an existing highly pure grade of coenzyme Q_{10}. Also known are the method which comprises using zinc as a reducing agent (Journal of Labelled Compounds, vol. 6, 1970, 66-75) and the method which comprises using vitamin C species (i.e. ascorbic acid or related compounds such as ascorbic acid, ascorbic acid palmitate, and ascorbic acid stearate) as reducing agents (WO 01/52822 A1).

However, the reduced coenzyme Q_{10} produced in such a manner cannot always be recovered in a highly pure state. For example, it is often obtained in the form of low-purity crystals,

semisolids or oil containing oxidized coenzyme Q_{10} and other impurities. When crystals of reduced coenzyme Q_{10} is recovered from an organic solvent solution containing reduced coenzyme Q_{10}, in particular, it is difficult to remove water-soluble impurities, particularly the reducing agent used for converting oxidized coenzyme Q_{10} into reduced coenzyme Q_{10} and/or impurities derived from such reducing agent and, therefore, the crystals obtained very often contain such water-soluble impurities and are of low purity.

DISCLOSURE OF THE INVENTION

In view of the foregoing, the present invention has an object to provide a purification method for removing impurities, in particular water-soluble impurities, contained in reduced coenzyme Q_{10}, and thereby producing high-quality reduced coenzyme Q_{10} in a convenient and efficient manner on an industrial scale production.

As a result of intensive investigations, the present inventors found that when an attempt was made to remove the water-soluble impurities remaining in reduced coenzyme Q_{10} by using water alone, particularly to remove the reducing agent and/or impurities derived from reducing agent, it was not always easy to decrease content of said impurities to at least to trace levels. It was also found that reduced coenzyme Q_{10} showed very poor wettability characteristics against water and thus it was very difficult to obtain slurry thereof having good properties. It was found, however, that the water-soluble impurities, in particular the reducing agent and/or impurities derived therefrom, remaining in reduced coenzyme Q_{10} could be removed efficiently with good operationality by washing reduced coenzyme Q_{10} (crystals or oil) with a water-soluble organic solvent or a mixed solvent composed of a water-soluble organic solvent and water. Based on these findings, the present invention has now completed.

Thus, the present invention provides a method of

purifying reduce coenzyme Q_{10}

which comprises washing crystals and/or oil of reduced coenzyme Q_{10} with a water-soluble organic solvent or a mixed solvent composed of a water-soluble organic solvent and water to thereby remove a water-soluble impurity from the crystals and/or oil of reduced coenzyme Q_{10}.

In accordance with the method of the present invention, the water-soluble impurities contained in reduced coenzyme Q_{10} can be conveniently and efficiently removed at least to a trace level and reduced coenzyme Q_{10} of very high quality can be obtained as a crystalline or an oily form.

In purifying oil of reduced coenzyme Q_{10}, it is also possible to crystallize it by cooling together with the solvent used for washing to recover the crystals formed, or to solidify of reduced coenzyme Q_{10} by contacting seed crystals to an oily form of reduced coenzyme Q_{10} at a temperature lower than the melting point thereof, to recover the crystals formed.

DETAILED DISCLOSURE OF THE INVENTION

In the following, the present invention is described in more detail.

The method of purifying reduced coenzyme Q_{10} according to the present invention is a method comprises washing crystalline and/or an oily form of reduced coenzyme Q_{10} with a water-soluble organic solvent or a mixed solvent composed of a water-soluble organic solvent and water to thereby remove a water-soluble impurity from the crystals and/or oil of reduced coenzyme Q_{10}.

Namely, in accordance with the invention, washing is carried out with a water-soluble organic solvent or a mixed solvent composed of a water-soluble organic solvent and water to conveniently and efficiently remove water-soluble impurities remaining in crystals and/or oil of reduced coenzyme Q_{10}, in particular the reducing agent and/or impurities derived from a reducing agent, which are to be mentioned later herein.

The water-soluble organic solvent used in the practice of the present invention is not particularly restricted provided that it is highly miscible with water, but includes alcohols, ethers, ketones, nitriles, amides, sulfur-containing compounds, fatty acids, and the like.

The alcohols may be used for the present invention are not particularly restricted but may be cyclic or acyclic, or saturated or unsaturated. Saturated ones are preferred, however. Generally, one containing 1 to 20 carbon atoms, preferably 1 to 12 carbon atoms, more preferably 1 to 6 carbon atoms, still more preferably 1 to 3 carbon atoms, and particularly preferably 2 or 3 carbon atoms, may favorably be used.

As specific examples of the alcohols, there may be mentioned methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, isobutyl alcohol, tert-butyl alcohol, 1-pentanol, 2-pentanol, 3-pentanol, 2-methyl-1-butanol, isopentyl alcohol, tert-pentyl alcohol, 3-methyl-2-butanol, neopentyl alcohol, 1-hexanol, 2-methyl-1-pentanol, 4-methyl-2-pentanol, 2-ethyl-1-butanol, 1-heptanol, 2-heptanol, 3-heptanol, 1-octanol, 2-octanol, 2-ethyl-1-hexanol, 1-nonanol, 3,5,5-trimethyl-1-hexanol, 1-decanol, 1-undecanol, 1-dodecanol, allyl alcohol, propargyl alcohol, benzyl alcohol, cyclohexanol, 1-methylcyclohexanol, 2-methylcyclohexanol, 3-methylcyclohexanol, 4-methylcyclohexanol, 2-methoxyethanol, 2-ethoxyethanol, 2-(methoxymethoxy)ethanol, 2-isopropoxyethanol, 2-buthoxyethanol, 2-(isopentyloxy)ethanol, 2-(hexyloxy)ethanol, furfuryl alcohol, 1-methoxy-2-propanol, 1-ethoxy-2-propanol, 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 2,3-butanediol, 1,5-pentanediol, 2-butane-1,4-diol, 2-methyl-2,4-pentanediol, 2-ethyl-1,3-hexanediol, diethylene glycol, triethylene glycol, tetraethylene glycol, polyethylene glycol,

dipropylene glycol, polypropylene glycol, glycerol, etc.

As monohydric alcohols, preferred ones which may be mentioned are methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, isobutyl alcohol, tert-butyl alcohol, 1-pentanol, 2-pentanol, 3-pentanol, 2-methyl-1-butanol, isopentyl alcohol, tert-pentyl alcohol, 3-methyl-2-butanol, neopentyl alcohol, 1-hexanol, 2-methyl-1-pentanol, 4-methyl-2-pentanol, 2-ethyl-1-butanol, 1-heptanol, 2-heptanol, 3-heptanol, 1-octanol, 2-octanol, 2-ethyl-1-hexanol, 1-nonanol, 1-decanol, 1-undecanol, 1-dodecanol, benzyl alcohol, cyclohexanol, 1-methylcyclohexanol, 2-methylcyclohexanol, 3-methylcyclohexanol, 4-methylcyclohexanol, 2-methoxyethanol, 2-ethoxyetanol, 2-(methoxymethoxy)ethanol, etc. More preferred are methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, isobutyl alcohol, tert-butyl alcohol, 1-pentanol, 2-pentanol, 3-pentanol, 2-methyl-1-butanol, isopentyl alcohol, tert-pentyl alcohol, 3-methyl-2-butanol, neopentyl alcohol, 1-hexanol, 2-methyl-1-pentanol, 4-methyl-2-pentanol, 2-ethyl-1-butanol, cyclohexanol, etc. Still more preferred are methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, isobutyl alcohol, tert-butyl alcohol, 1-pentanol, 2-pentanol, 3-pentanol, 2-methyl-1-butanol, isopentyl alcohol, tert-pentyl alcohol, 3-methyl-2-butanol, neopentyl alcohol, etc. Particularly preferred are methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, isobutyl alcohol, 2-methyl-1-butanol, isopentyl alcohol, etc. Further preferred are methanol, ethanol, 1-propanol, 2-propanol, etc., and most preferred is ethanol.

As the dihydric alcohol, preferred ones which may be mentioned are 1,2-ethanediol, 1,2-propandiol, 1,3-propandiol, 2-butane-1,4-diol, 2-methyl-2,4-pentanediol, 2-ethyl-1,3-hexanediol, diethylene glycol, triethylene, glycol, tetraethylene glycol, polyethylene glycol,

dipropylene glycol, polypropylene glycol, etc., and most preferred are 1,2-propanediol and polyethylene glycol.

As the trihydric alcohol, glycerol may be preferably used.

The ethers are not particularly restricted, and may be cyclic or acyclic, or saturated or unsaturated. But in general, saturated ones are preferably used. Generally, ones containing 3 to 20 carbon atoms, and preferably 4 to 12 carbon atoms and more preferably 4 to 8 carbon atoms are used.

As specific examples, there may be mentioned, for example, diethyl ether, methyl tert-butyl ether, dipropyl ether, diisopropyl ether, dibutyl ether, dihexyl ether, ethyl vinyl ether, butyl vinyl ether, anisol, phenetole, butyl phenyl ether, methoxytoluene, dioxane, furan, 2-methylfuran, tetrahydrofuran, tetrahydropyran, ethylene glycol dimethyl ether, ethylene glycol diethyl ether, ethylene glycol dibutyl ether, ethylene glycol monomethyl ether, ethylene glycol monoethyl ether, ethylene glycol monobutyl ether, diethylene glycol monomethyl ether, diethylene glycol monoethyl ether, diethylene glycol monobutyl ether, triethylene glycol monomethyl ether, dipropylene glycol monomethyl ether, dipropylene glycol monoethyl ether, tripropylene glycol monomethyl ether, etc.

Preferred are diethyl ether, methyl tert-butyl ether, dipropyl ether, diisopropyl ether, dibutyl ether, dihexyl ether, anisol, phenetole, butyl phenyl ether, methoxytoluene, dioxane, 2-methylfuran, tetrahydrofuran, tetrahydropyran, ethylene glycol dimethyl ether, ethylene glycol diethyl ether, ethylene glycol dibutyl ether, ethylene glycol monomethyl ether, ethylene glycol monoethyl ether, etc. More preferred are diethyl ether, methyl tert-butyl ether, anisol, dioxane, tetrahydrofuran, ethylene glycol monomethyl ether, ethylene glycol monoethyl ether, etc. Still more preferred are diethyl ether, methyl tert-butyl ether, anisol, dioxane, tetrahydrofuran, etc., and particularly preferred are dioxane

and tetrahydrofuran.

The ketones are not particularly restricted, and ones having 3 to 6 carbon atoms are preferably used. As specific examples, there may be mentioned, for example, acetone, methyl ethyl ketone, methyl butyl ketone, methyl isobutyl ketone, etc. Preferred are acetone and methyl ethyl ketone, and most preferred is acetone.

The nitriles are not particularly restricted, and may be cyclic or acyclic, or saturated or unsaturated. However, saturated ones are preferably used. Generally, ones containing 2 to 20 carbon atoms, preferably 2 to 12 carbon atoms, and more preferably 2 to 8 carbon atoms are used.

As specific examples, there may be mentioned, for example, acetonitrile, propiononitrile, malononitrile, butyronitrile, isobutyronitrile, succinonitrile, valeronitrile, glutaronitrile, hexanenitrile, heptylcyanide, octylcyanide, undecanenitrile, dodecanenitrile, tridecanenitrile, pentadecanenitrile, stearonitrile, chloroacetonitrile, bromoacetonitrile, chloropropiononitrile, bromopropiononitrile, methoxyacetonitrile, methyl cyanoacetate, ethyl cyanoacetate, tolunitrile, benzonitrile, chlorobenzonitrile, bromobenzonitrile, cyanobenzoic acid, nitrobenzonitrile, anisonitrile, phthalonitrile, bromotolunitrile, methyl cyanobenzoate, methoxybenzonitrile, acetylbenzonitrile, naphthonitrile, biphenylcarbonitrile, phenylpropiononitrile, phenylbutyronitrile, methylphenylacetonitrile, diphenylacetonitrile, naphthylacetonitrile, nitrophenylacetonitrile, chlorobenzylcyanide, cyclopropanecarbonitrile, cyclohexanecarbonitrile, cycloheptanecarbonitrile, phenylcyclohexanecarbonitrile, tolylcyclohexanecarbonitrile, etc.

Preferred are acetonitrile, propiononitrile, butyronitrile, isobutyronitrile, succinonitrile, valeronitrile, methyl cyanoacetate, ethyl cyanoacetate,

benzonitrile, tolunitrile and chloropropiononitrile. More preferred are acetonitrile, propiononitrile, butyronitrile and isobutyronitrile, and most preferred is acetonitrile.

As the amides, there may be mentioned, for example, formamide, N-methylformamide, N, N-dimethylformamide, N,N-dimethylacetoamide, N-methylpyrrolidone, etc.

As the sulfur-containing compounds, there may be mentioned, for example, dimethyl sulfoxide, sulfolane, etc.

As the fatty acids, there may be mentioned, for example, formic acid, acetic acid, propionic acid, etc. Preferred are formic acid and acetic acid, and particularly preferred is acetic acid.

Among the above water-soluble solvents, alcohols, ethers, ketones, nitriles are preferred, alcohols and ketones are more preferred, monohydric alcohols containing 1 to 3 carbon atoms and acetone are still more preferred, and ethanol is most preferred.

The water-soluble organic solvents mentioned above may be used singly or in the form of a mixed solvent composed of two or more species. Furthermore, they may also be favorably used in the form of mixed solvents in combination with water. From the viewpoint of liquid characteristics and/or washing effects, the use of a mixed solvent composed of a water-soluble organic solvent and water is preferred.

When a mixed solvent composed of a water-soluble organic solvent and water is used, the concentration of the water-soluble organic solvent contained in the mixed solvent is not particularly restricted but, from the viewpoint for obtaining favorable liquid characteristics and washing effects, it is preferably not less than about 5 w/w%, more preferably not less than about 7 w/w%, still more preferably not less than about 10 w/w%, particularly preferably not less than about 20 w/w%, and most preferably not less than about 30 w/w%.

In cases where the product is used for foods or drugs, for instance, ethanol, 1,2-propanediol, polyethylene glycol

(preferably polyethylene glycol having a molecular weight of 300 to 1000), glycerol and the like are suitable, and ethanol is particularly suitable. Needless to say, these solvents may be favorably used as a mixed solvent of two or more of them or as a mixed solvent in combination with water.

In the practice of the invention, a water-insoluble organic solvent may be used in combination with any of the water-soluble solvents mentioned above within the range that no substantial adverse effect will be caused. Such water-insoluble organic solvent includes hydrocarbons, fatty acid esters and the like, which are to be mentioned later herein.

The reduced coenzyme Q_{10} to be used in the practice of the invention can be obtained by conventional methods such as synthesis, fermentation, or extraction from a natural source. Preferred is one obtainable by reduction of oxidized coenzyme Q_{10} contained in reduced coenzyme Q_{10} or of oxidized coenzyme Q_{10}. More preferred is one obtainable by utilizing the reduction reaction according to the invention, which is to be mentioned later herein.

The purification method of the invention can be applied to reduced coenzyme Q_{10} containing a relatively large amount of oxidized coenzyme Q_{10}, but it is especially effective in purifying high-purity reduced coenzyme Q_{10} prepared by the reduction method to be mentioned later herein, or the like method. Needless to say, the reduced coenzyme Q_{10} to be purified may be in the form of either crystals or oil. The term "crystal(s)" of reduced coenzyme Q_{10} as used herein also includes, within the meaning thereof, a solid obtainable by concentrating a reduced coenzyme Q_{10}-containing solution to dryness by distilling off the solvent, a solid resulting from solidification of oil of reduced coenzyme Q_{10}, and the like.

The water-soluble impurity to be removed in accordance with the present invention is not particularly restricted but includes, among others, the reducing agents used in the step of reducing oxidized coenzyme Q_{10}, which are to be mentioned

later herein, and/or impurities derived from reducing agents. As the reducing agents and/or impurities derived from reducing agents, there may be mentioned, for example, hyposulfurous acid or salts thereof, and hydrogensulfites as byproducts derived from said hyposulfurous acid or salts thereof; ascorbic acid or related compounds thereof, and dehydroascorbic acid, 2,3-diketogulonic acid and oxalic acid as byproducts derived from said ascorbic acid or related compounds thereof; salts generated as byproducts from iron or zinc; and the like.

The method of washing is not particularly restricted but generally comprises bringing the crystals and/or oil of reduced coenzyme Q_{10} into contact with the above-mentioned water-soluble organic solvent or the mixed solvent composed of the water-soluble organic solvent and water in a vessel since the amount of the solvent for washing can then be decreased. This contact is preferably established by dispersing the crystals and/or oil of reduced coenzyme Q_{10} in the water-soluble organic solvent or the mixed solvent composed of the water-soluble organic solvent and water, and particularly preferably suspending and/or emulsifying the crystals and/or oil of reduced coenzyme Q_{10} in the water-soluble organic solvent or the mixed solvent composed of the water-soluble organic solvent and water to a sufficient extent.

Preferably, the washing is carried out under forced flowing. From the quality improvement viewpoint, the flowing is preferably effected by a power required for stirring per unit volume of generally not less than about 0.01 kW/m^3, preferably not less than about 0.1 kW/m^3, and more preferably not less than about 0.3 kW/m^3. The above forced flowing is generally provided by rotation of a stirring blade(s). If the above flowing is attained, however, it is not always necessary to use a stirring blade(s). For example, the circulation of the liquid or the like procedure may be utilized.

The concentration of reduced coenzyme Q_{10} during washing of crystals and/or oil of reduced coenzyme Q_{10} is not

particularly restricted but, from the viewpoint of attaining favorable liquid characteristics, the weight of reduced coenzyme Q_{10} relative to the weight of the washing solvent at the time of completion of washing is preferably about not more than about 30 w/w%, more preferably not more than about 20 w/w%, still more preferably not more than about 15 w/w%, particularly preferably not more than about 13 w/w%, and most preferably not more than about 10 w/w%. By maintaining the above concentration, it becomes possible to realize more favorable washing with sufficient operationality for an industrial scale production. From the productivity viewpoint, the lower limit to that concentration is preferably about 1 w/w%, and more preferably about 2 w/w%.

The time of washing may vary depending on species of the water-soluble organic solvent or a mixed solvent composed of a water-soluble organic solvent and water, the proportion thereof, the amount of the washing solvent and so on, hence cannot be absolutely specified. Generally, however, the washing can be completed within a time not longer than 10 hours, preferably not longer than 5 hours, more preferably not longer than 2 hours, still more preferably not longer than 1 hour, particularly preferably not longer than 30 minutes, and most preferably not longer than 10 minutes.

The washing temperature may vary depending on the amount, composition and/or composition proportion of the solvent for washing (water-soluble organic solvent or a mixed solvent composed of a water-soluble organic solvent and water), the quality or purity of the reduced coenzyme Q_{10} to be purified and so forth, hence cannot be absolutely specified. But when crystals of reduced coenzyme Q_{10} is used, the upper limit is generally not higher than about 50°C, preferably not higher than about 45°C, more preferably not higher than about 40°C, and still more preferably not higher than about 35°C, and the lower limit is not lower than about -10°C, preferably not lower than about -5°C, still more preferably not lower than about 0°C.

Generally, the washing can be favorably carried out within the range of about 0°C to 40°C. On the other hand, when oil of reduced coenzyme Q_{10} is used, the lower limit is not lower than the melting temperature of reduced coenzyme Q_{10}, preferably not lower than about 40°C, more preferably not lower than about 45°C, still more preferably not lower than about 50°C, and particularly preferably not lower than about 60°C, and the upper limit is not higher than about 100°C, preferably not higher than about 90°C, more preferably not higher than about 80°C, and still more preferably not higher than about 70°C.

By washing crystals and/or oil of reduced coenzyme Q_{10} in accordance with the above mentioned method, water-soluble impurities contained in the crystals and/or oil of reduced coenzyme Q_{10} can be transferred into a water-soluble organic solvent or a mixed solvent composed of a water-soluble organic solvent and water, and thereby the water-soluble impurities can be removed from said the crystals and/or oil of reduced coenzyme Q_{10}.

In cases where crystals of reduced coenzyme Q_{10} is purified, the reduced coenzyme Q_{10} from which water-soluble impurities were removed can be recovered as a wet product, for example, by centrifugation, pressure filtration, vacuum filtration and/or the like procedure to remove the above-mentioned solvent used in the washing, if necessary followed by cake washing. Moreover, they can be recovered also as a dry product by further charging the wet product in a reduced pressure drier (vacuum drier) internally purged with an inert gas and drying the same under reduced pressure. The recovery as a dry product is preferred.

Furthermore, in cases where oil of reduced coenzyme Q_{10} is purified by this method, it is possible to recover reduced coenzyme Q_{10} as an oily form by separating a solvent used for washing from wash mixture (oil of reduced coenzyme Q_{10} + a solvent(s) used for washing). On the other hand, it is also possible to recover reduced coenzyme Q_{10} as a crystalline form

by cooling the wash mixture as it is. The cooling temperature in the case where reduced coenzyme Q_{10} is recovered as a crystalline form is not particularly restricted but is generally lower than about 50°C, preferably lower than about 48°C, more preferably lower than about 45°C, and still more preferably lower than about 40°C. The lower limit is the solidification temperature of the system and generally is not lower than about 0°C. In this case, the reduced coenzyme Q_{10} from which water-soluble impurities were removed can be recovered despite the presence of water-soluble impurities in a wash mixture.

In cases where reduced coenzyme Q_{10} was recovered as an oily form, it is also possible to favorably solidify the oil of reduced coenzyme Q_{10} by contacting seed crystals (reduced coenzyme Q_{10} own crystals) to the oil of reduced coenzyme Q_{10}, particularly by contacting seed crystals (reduced coenzyme Q_{10} own crystals) to the oil of reduced coenzyme Q_{10} at a temperature lower than the melting temperature. In this case, a solid may be obtained by forming the above oily product into a desired form after decreasing the temperature of the oily product to below the melting temperature thereof and contacting with the seed crystals. The contact with the crystals may be performed either before or after said formation from the oily product. The solidification temperature is not particularly restricted as long as it is lower than the melting temperature. Desirably, however, it is not lower than 0°C.

By recovering crystals (including solid) of reduced coenzyme Q_{10} from oil of reduced coenzyme Q_{10} in the above manner, the reagent and time losses can be avoided and crystals of reduced coenzyme Q_{10} (including solid) can be favorably obtained in a high yield.

The weight content of water-soluble impurities in reduced coenzyme Q_{10}, when purified in the above manner, can be decreased generally to 0.15% or below, preferably 0.10% or below, more preferably 0.08% or below. Thus, reduced coenzyme Q_{10} with very

high quality can be obtained.

It is particularly preferable to perform the above purification procedure in a deoxygenated atmosphere. By doing so, it becomes possible to minimize the formation of oxidized coenzyme Q_{10} as a byproduct and thus do the washing more efficiently.

The deoxygenated atmosphere can be attained by inert gas substitution, pressure reduction, boiling, or a combination of these. It is appropriate to perform at least inert gas substitution, namely to use an inert gas atmosphere. As the inert gas, there may be mentioned, for example, nitrogen gas, helium gas, argon gas, hydrogen gas, carbon dioxide gas or the like. Nitrogen gas is preferred, however.

Next, a method of synthesizing reduced coenzyme Q_{10}, which is suited for use in the practice of the invention, namely a method of reducing oxidized coenzyme Q_{10} into reduced coenzyme Q_{10}, is described.

Reduced coenzyme Q_{10} which can be used in the practice of the invention can be obtained by conventional methods such as synthesis, fermentation, or extraction from a natural source, as already mentioned hereinabove. They can be obtained preferably by reducing oxidized coenzyme Q_{10}, such as an existing highly pure coenzyme Q_{10}, or a mixture of oxidized coenzyme Q_{10} and reduced coenzyme Q_{10} with a common reducing agent. First, the method of reducing oxidized coenzyme Q_{10} is described.

Since reduced coenzyme Q_{10} is apt to be oxidized by molecular oxygen to give oxidized coenzyme Q_{10} as a byproduct, a solvent with high protective effect against oxidation is preferably used as the solvent in the step of reduction. Preferably, at least one species selected from among hydrocarbons, fatty acid esters, ethers, and nitriles is used as such solvent. Hydrocarbons are most preferred among them.

The hydrocarbons are not particularly restricted, but there may be mentioned, for example, aliphatic hydrocarbons,

aromatic hydrocarbons, halogenated hydrocarbons, etc. Preferred are aliphatic hydrocarbons and aromatic hydrocarbons, and more preferred are aliphatic hydrocarbons.

The aliphatic hydrocarbons are not particularly restricted, and may be cyclic or acyclic, or saturated or unsaturated. However, generally they contain 3 to 20 carbon atoms, and preferably 5 to 12 carbon atoms.

As specific examples, there may be mentioned, for example, propane, butane, isobutane, pentane, 2-methylbutane, cyclopentane, 2-pentene, hexane, 2-methylpentane, 2,2-dimethylbutane, 2,3-dimethylbutane, methylcyclopentane, cyclohexane, 1-hexene, cyclohexene, heptane, 2-methylhexane, 3-methylhexane, 2,3-dimethylpentane, 2,4-dimethylpentane, methylcyclohexane, 1-heptene, octane, 2,2,3-trimethylpentane, isooctane, ethylcyclohexane, 1-octene, nonane, 2,2,5-trimethylhexane, 1-nonene, decane, 1-decene, p-menthane, undecane, dodecane, etc.

Among them, saturated aliphatic hydrocarbons having 5 to 8 carbon atoms are preferred, and preferably used are pentane, 2-methylbutane and cyclopentane, which have 5 carbon atoms (referred to as "pentanes"); hexane, 2-methylpentane, 2,2-dimethylbutane, 2,3-dimethylbutane, methylcyclopentane, cyclohexane, which have 6 carbon atoms (referred to as "hexanes"); heptane, 2-methylhexane, 3-methylhexane, 2,3-dimethylpentane, 2,4-dimethylpentane, methylcyclohexane, which have 7 carbon atoms (referred to as "heptanes"); octane, 2,2,3-trimethylpentane, isooctane, ethylcyclohexane, which have 8 carbon atoms (referred to as octanes); and a mixture of these. In particular, the above heptanes are still more preferred since they have a tendency to show a very high protective effect against oxidization, and heptane is most preferred.

The aromatic hydrocarbons are not particularly restricted, but generally they contain 6 to 20 carbon atoms, preferably 6 to 12 carbon atoms, and more preferably 7 to 10

carbon atoms. As specific examples, there may be mentioned, for example, benzene, toluene, xylene, o-xylene, m-xylene, p-xylene, ethylbenzene, cumene, mesitylene, tetralin, butylbenzene, p-cymene, cyclohexylbenzene, diethylbenzene, pentylbenzene, dipentylbenzene, dodecylbenzene, styrene, etc. Preferred are toluene, xylene, o-xylene, m-xylene, p-xylene, ethylbenzene, cumene, mesitylene, tetralin, butylbenzene, p-cymene, cyclohexylbenzene, diethylbenzene and pentylbenzene. More preferred are toluene, xylene, o-xylene, m-xylene, p-xylene, cumene and tetralin, and most preferred is cumene.

The halogenated hydrocarbons are not particularly restricted, and may be cyclic or acyclic, or saturated or unsaturated. However, acyclic halogenated hydrocarbons are preferably used. More preferred are chlorinated hydrocarbons and fluorinated hydrocarbons, and chlorinated hydrocarbons are particularly preferred. Additionally, ones containing 1 to 6 carbon atoms, preferably 1 to 4 carbon atoms, and more preferably 1 to 2 carbon atoms are favorably used.

As specific examples, there may be mentioned, for example, dichloromethane, chloroform, carbon tetrachloride, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,1,1,2-tetrachloroethane, 1,1,2,2-tetrachloroethane, pentachloroethane, hexachloroethane, 1,1-dichloroethylene, 1,2-dichloroethylene, trichloroethylene, tetrachloroethylene, 1,2-dichloropropane, 1,2,3-trichloropropane, chlorobenzene, 1,1,1,2-tetrafluoroethane, etc.

Preferred are dichloromethane, chloroform, carbon tetrachloride, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,1-dichloroethylene, 1,2-dichloroethylene, trichloroethylene, chlorobenzene and 1,1,1,2-tetrafluoroethane. More preferred are dichloromethane, chloroform, 1,2-dichloroethylene, trichloroethylene, chlorobenzene and

1,1,1,2-tetrafluoroethane.

The fatty acid esters are not particularly restricted, but there may be mentioned, for example, propionates, acetates, formates, etc. Preferred are acetates and formates, and more preferred are acetates. Ester functional groups thereof are not particularly restricted, but include alkyl esters having 1 to 8 carbon atoms, aralkyl esters having 7 to 12 carbon atoms. Preferred are alkyl esters having 1 to 6 carbon atoms, and more preferred are alkyl esters having 1 to 4 carbon atoms are used.

As the propionates, there may be mentioned, for example, methyl propionate, ethyl propionate, butyl propionate, isopentyl propionate, etc. Preferred is ethyl propionate.

As the acetates, there may be mentioned, for example, methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, isobutyl acetate, sec-butyl acetate, pentyl acetate, isopentyl acetate, sec-hexyl acetate, cyclohexyl acetate, benzyl acetate, etc. Preferred are methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, isobutyl acetate, sec-butyl acetate, pentyl acetate, isopentyl acetate, sec-hexyl acetate and cyclohexyl acetate. More preferred are methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate and isobutyl acetate. Most preferred is ethyl acetate.

As the formates, there may be mentioned, for example, methyl formate, ethyl formate, propyl formate, isopropyl formate, butyl formate, isobutyl formate, sec-butyl formate, pentyl formate, etc. Preferred are methyl formate, ethyl formate, propyl formate, butyl formate, isobutyl formate and pentyl formate, and most preferred is ethyl formate.

As the ethers and nitriles, there may be mentioned such ethers and nitriles as described hereinabove.

In selecting the solvent to be used from among those mentioned above, the following factors are preferably taken into consideration: such properties as boiling point and viscosity (e.g. boiling point (about 30 to 150°C at 1 atm)

allowing appropriate warming for increasing the solubility and facilitating solvent removal by drying from wet crystals and/or solvent recovery from the filtrate after crystallization, etc.), an adequate melting point (not higher than about 20°C, preferably not higher than about 10°C, and more preferably not higher than about 0°C) hardly allowing solidification during handling at room temperature and upon cooling to a level below room temperature, and a low level of viscosity (about 10 cp or below at 20°C). From the industrial operation viewpoint, those that are hardly volatile at ordinary temperature are preferred. Generally preferred are those having a boiling point of, for example, about 80°C or higher, and more preferably about 90°C or higher.

Among the above-mentioned solvents, those solvents which are low in miscibility with water are particularly preferably used as the solvent in carrying out the reduction reaction. They promote extraction/removal of the reducing agent (to be mentioned later) and impurities derived from the reducing agent into the aqueous phase and efficient purification/recovery of reduced coenzyme Q_{10}.

Reduced coenzyme Q_{10} tends to become more resistant to oxidation as the concentration thereof in solution increases. Reduced coenzyme Q_{10} is highly soluble in the solvents mentioned above and, from this viewpoint as well, the above solvents are suited for protection against oxidation. The concentration of reduced coenzyme Q_{10} which is preferred for the protection against oxidation may vary depending on the solvent species, hence cannot be absolutely specified. Generally, however, the concentration of reduced coenzyme Q_{10} in the solvents mentioned above is not lower than about 1 w/w%, preferably not lower than about 2 w/w%. The upper limit is not particularly restricted but, from the practical operationality viewpoint, it is about 400 w/w%, preferably about 200 w/w%, more preferably about 100 w/w%, and still more preferably about 50 w/w%.

Thus, when the above solvents are used, the undesirable

oxygen-involved side reactions are minimized through the reduction step.

The reduction reaction can be carried out in one of the above solvents using, as the reducing agent, a metal hydride compound, iron (metallic iron or a salt-form iron), zinc (metallic zinc), hyposulfurous acid or a salt thereof, ascorbic acid or related compounds thereof, or the like.

The metal hydride compound is not particularly restricted but includes sodium borohydride, lithium aluminum hydride, etc. The amount of the metal hydride compound to be used may vary depending on the metal hydride compound species, hence cannot be absolutely specified. Generally, however, an amount of 1 to 3 times the theoretical hydrogen equivalent is suitable for carrying out the reduction reaction.

The reduction using iron or zinc is generally carried out using an acid. The acid is not particularly restricted but includes fatty acids such as acetic acid, sulfonic acids such as methanesulfonic acid, and inorganic acids such as hydrochloric acid and sulfuric acid, and the like. Inorganic acids are preferred, and sulfuric acid is more preferred.

The amount of iron to be used is not particularly restricted but an amount of about 1/5 by weight or more relative to the weight of reduced coenzyme Q_{10} charged is appropriate for carrying out the reaction. The upper limit is not particularly restricted but, from the economic viewpoint, among others, it is about 2 times by weight or below. Iron may be used not only in a metallic form but also in the form of a salt such as iron(II) sulfate.

The amount of zinc to be used is not particularly restricted but an amount of about 1/10 by weight or more relative to the weight of reduced coenzyme Q_{10} charged is appropriate for carrying out the reaction. The upper limit is not particularly restricted but, for the economic viewpoint, among others, it is not more than about 2 times by weight.

The hyposulfurous acid or the salt thereof is not

particularly restricted but generally used in the form of an alkali metal salt (lithium salt, sodium salt, potassium salt, and the like), alkaline earth metal salt (magnesium salt, calcium salt, and the like), or ammonium salt, of hyposulfurous acid, for instance. Alkali metal salts, such as the lithium salt, sodium salt, and potassium salt, are preferred, and the sodium salt is more preferred.

The amount of the hyposulfurous acid or the salt thereof to be used is not particularly restricted but, generally, it is not less than about 1/5 by weight, preferably not less than about 2/5 by weight, and more preferably about 3/5 by weight, relative to the weight of reduced coenzyme Q_{10} charged. A larger amount will not cause any trouble but is economically unfavorable. Thus, an amount not exceeding about 2 times by weight, preferably not exceeding the same weight, is employed. The reduction reaction can be appropriately carried out in an amount within the range from about 2/5 by weight to about the same weight.

The ascorbic acid and related compounds thereof are not particularly restricted, and include, for example, not only ascorbic acid, but also rhamno-ascorbic acid, arabo-ascorbic acid, gluco-ascorbic acid, fuco-ascorbic acid, glucohepto-ascorbic acid, xylo-ascorbic acid, galacto-ascorbic acid, gulo-ascorbic acid, allo-ascorbic acid, erythro-ascorbic acid, 6-desoxyascorbic acid, and the like related compounds, and may be ester forms or salts of these. Furthermore, these may be L-form, D-form or racemic form. More specifically, there may be mentioned, for example, L-ascorbic acid, L-ascorbyl palmitate, L-ascorbyl stearate, D-arabo-ascorbic acid, etc.

In producing the reduced coenzyme Q_{10}, any of the above-mentioned ascorbic acid and related compounds thereof may be favorably used. However, the water-soluble ones are favorably used in particular among the above-mentioned ascorbic acid or related compounds thereof in view of ease of

separation from the generated reduced coenzyme Q_{10}, etc. And most preferred are a free form of L-ascorbic acid, D-arabo-ascorbic acid and the like in view of the ready availability, price, etc.

The amount of the ascorbic acid or a related compound thereof to be used is not particularly restricted but may be at such a level that is effective for converting oxidized coenzyme Q_{10} to reduced coenzyme Q_{10}. Generally, it is used in an amount of not less than 1 mole, preferably not less than 1.2 moles, per mole of reduced coenzyme Q_{10}. The upper limit is not particularly restricted but, taking economic viewpoint into consideration, it is generally 10 moles, preferably 5 moles, and more preferably 3 moles, on the same basis.

The reducing agents mentioned above and/or compounds derivable therefrom are mostly soluble in water. When hyposulfurous acid or a salt thereof is used, for instance, a hydrogensulfite is formed as a byproduct. When ascorbic acid or a related compound thereof is used, dehydroascorbic acid is formed as a byproduct, and further 2,3-diketogulonic acid and oxalic acid are formed as byproducts from dehydroascorbic acid. Furthermore, when iron or zinc is used, salts (e.g. iron chloride or zinc chloride, which may be generated as a byproduct when hydrochloric acid is used) are formed as byproducts after reduction. As mentioned hereinabove, all of these reducing agents and/or compounds derived therefrom can be efficiently removed by using the purification method of the present invention, whereby high-quality reduced coenzyme Q_{10} can be obtained.

Among the reducing agents mentioned above, zinc, hyposulfurous acid or a salt thereof, and ascorbic acid or a related compound thereof are more preferred, and hyposulfurous acid or a salt thereof (specifically, a hyposulfurous acid salt) and ascorbic acid or a related compound thereof are especially preferred, from the viewpoint of reducing ability, yield, quality or the like.

In the reduction reaction, such an alcohol as mentioned above and/or water can be appropriately used in combination. Combined use of water is suitable especially when iron, zinc or hyposulfurous acid or a salt thereof is used as the reducing agent. When a metal hydride compound or ascorbic acid or a related compound thereof is used as the reducing agent, an alcohol can be preferably used in combination. The combined use of water and/or an alcohol makes it possible to show the characteristics of these and contributes to improvements in reaction rate, yield and the like.

In the following, preferred modes of the reduction method are described in detail.

The reduction using the above-mentioned hyposulfurous acid or a salt thereof is preferably carried out in a mixed solvent system composed of water together with at least one organic solvent selected from among the above-mentioned hydrocarbons, fatty acid esters, ethers, and nitriles (preferably hydrocarbons, more preferably aliphatic hydrocarbons, still more preferably heptanes, and particularly preferably heptane).

On that occasion, the reaction is carried out generally at a pH of not higher than 7, preferably at pH 3 to 7, and more preferably at pH 3 to 6, from the yield and/or other viewpoint. The pH can be adjusted using an acid, for example a mineral acid such as hydrochloric acid or sulfuric acid, or a base, for example an alkali metal hydroxide such as sodium hydroxide.

In the reduction using hyposulfurous acid or a salt thereof, the amount of water to be used is not particularly restricted but may be such that a proper amount of hyposulfurous acid or a salt thereof, namely the reducing agent, can be dissolved. Generally, it is advisable to adjust the weight of the hyposulfurous acid or the salt relative to water to a level not more than about 30 w/w%, for instance, and preferably not more than about 20 w/w%. From the productivity viewpoint, among others, the amount of water is generally not less than about

1 w/w%, preferably not less than about 5 w/w%, and more preferably not less than about 10 w/w%.

The reduction using the above mentioned ascorbic acid or a related compound thereof can also be carried out using a solvent highly miscible with water as selected from among the above-mentioned hydrocarbons, fatty acid esters, ethers, and nitriles, in particular a highly water-miscible ether or nitrile, more specifically tetrahydrofuran, dioxane, acetonitrile, or the like. The use of such an alcohol and/or a ketone as mentioned above (preferably a highly water-miscible alcohol and/or ketone (specifically, a monohydric or dihydric (preferably monohydric) alcohol containing 1 to 5 carbon atoms, preferably 1 to 4 carbon atoms, more preferably 1 to 3 carbon atoms and/or such a ketone as acetone or methyl ethyl ketone)) is particularly preferred. Namely in the reduction reaction using ascorbic acid or a related compound, use of highly-miscible organic solvent is preferred.

The reduction using ascorbic acid or a related compound thereof can be carried out in the presence of a reaction promoter such as a basic substance or a hydrogensulfite salt in view of lowering the reaction temperature, shortening the reaction time and the like.

The above-mentioned basic substance is not particularly restricted but may be either an inorganic compound or an organic compound. The inorganic compound is not particularly restricted but includes hydroxides, carbonates and hydrogencarbonates of metals (preferably alkali metals, alkaline earth metals, etc.), ammonia, and the like. As typical examples thereof, there may be mentioned alkali metal hydroxides such as sodium hydroxide, alkali metal carbonates such as sodium carbonate, alkali metal hydrogencarbonates such as sodium hydrogencarbonate, alkaline earth metal carbonates such as magnesium carbonate, and the like. The organic compound is not particularly restricted but includes amines such as triethylamine, and the like.

Among the basic substances specifically mentioned above, weakly basic substances (weak base or weak alkali), for example such inorganic compounds as metal (preferably alkali metal, alkaline earth metal or the like) carbonates and hydrogencarbonates, and ammonia, and such organic compounds as triethylamine and like amines, are preferably used. Those weakly basic inorganic compounds mentioned above are more preferred.

As preferred species of the hydrogensulfite salt, there may be mentioned, for example, alkali metal hydrogensulfites such as sodium hydrogensulfite, and the like.

The amount of the above-mentioned reaction promoter is not particularly restricted but may be such that the reaction promoting effect of the reaction prompter can be produced to an expected extent (Namely, an effective amount). Taking economical viewpoint into consideration as well, it is generally not more than about 20 moles, preferably not more than about 10 moles, more preferably not more than about 5 moles, and still more preferably not more than about 2 moles, per mole of the ascorbic acid or a related compound thereof. The lower limit is not particularly restricted but generally is about 0.01 moles or higher, preferably about 0.05 moles or higher, more preferably about 0.1 moles or higher, and still more preferably about 0.2 moles or higher, on the same basis.

The reduction reaction is preferably carried out under forced flowing. The flowing is preferably effected by a power required for stirring per unit volume of generally not less than about 0.01 kW/m^3, preferably not less than about 0.1 kW/m^3, and more preferably not less than about 0.3 kW/m^3. The above forced flowing is generally provided by rotation of a stirring blade(s). If the above flowing is attained, however, it is not always necessary to use a stirring blade(s). For example, the circulation of the liquid or the like procedure may be utilized.

The reduction reaction temperature may vary depending on the reducing agent species and/or the amount thereof, hence

cannot be absolutely specified. In the case of reduction using hyposulfurous acid or a salt thereof, for instance, the reaction can be carried out generally at about 100°C or below, preferably at about 80°C or below, and more preferably at about 60°C or below. The lower limit is the solidification temperature of the system. The reaction can be smoothly carried out at about 0 to about 100°C, preferably at about 0 to about 80°C, and more preferably at about 0 to about 60°C. The reduction using the ascorbic acid or a related compound thereof is carried out generally at about 30°C or above, preferably at about 40°C or above, and more preferably at about 50°C or above. The upper limit is the boiling point of the system. Generally, the reaction can be carried out at about 30 to about 150°C, preferably at about 40 to about 120°C, and more preferably at about 50 to about 100°C.

The reaction concentration is not particularly restricted but the weight of oxidized coenzyme Q_{10} relative to the weight of the solvent is generally not less than about 1 w/w%, preferably not less than about 2 w/w%, more preferably not less than about 3 w/w%, still more preferably not less than about 5 w/w%, particularly preferably not less than about 10 w/w%, and most preferably not less than about 15 w/w%. The upper limit is not particularly restricted but generally is about 60 w/w%, preferably about 50 w/w%, more preferably about 40 w/w%, and still more preferably about 30 w/w%. Generally, the reaction may be favorably carried out at about 2 to about 30 w/w%, preferably at about 5 to about 30 w/w%, and more preferably at about 10 to about 30 w/w%.

The reduction reaction can be driven to completion generally within 48 hours, preferably within 24 hours, more preferably within 10 hours, and still more preferably within 5 hours.

The oil of reduced coenzyme Q_{10} may also be obtained by removing the aqueous phase after reduction, in water, of oil of oxidized coenzyme Q_{10} using the above-mentioned reducing

agent.

In this case, the reduction is carried out generally at a temperature of about 45°C or above, preferably at about 48°C or above, and more preferably at about 50°C of above, although the temperature depends on the purity of reduced coenzyme Q_{10} and other factors. The upper limit is the boiling point of the system and generally is about 100°C or below, preferably about 80°C or below, and more preferably about 60°C or below.

This method to reduce oil of oxidized coenzyme Q_{10} in water makes it possible to synthesize reduced coenzyme Q_{10} while avoiding the waste of time, the use of an expensive production apparatus and the increase in volume due to separation and concentration of the organic solvent.

The above reduction reaction and the after-treatment (separation of the organic phase) are very favorably carried out in a deoxygenated atmosphere, and it was also found that the operation under such atmosphere greatly contributed to the improvement in yield at the reduction reaction and the decrease of the amount of the reducing agent especially in the reduction reaction using hyposulfurous acid or a salt thereof. The deoxygenated atmosphere can be attained by inert gas substitution, pressure reduction, boiling, or a combination of these. It is appropriate to perform at least inert gas substitution, namely to use an inert gas atmosphere. The inert gas may be, for example, nitrogen gas, helium gas, argon gas, hydrogen gas, carbon dioxide gas, or the like. Nitrogen gas is preferred, however.

Now, the crystals of reduced coenzyme Q_{10} to be used in the purification method of the present invention are described. For the practice of the invention, the crystals which may be available are those crystals obtainable by crystallization from or concentration to dryness of a solution containing reduced coenzyme Q_{10}, the already existing crystals of reduced coenzyme Q_{10}, and the like. The solid resulting from solidification of oil of reduced coenzyme Q_{10} may also be used. Preferred are the

crystals of reduced coenzyme Q_{10} obtainable by crystallization or concentration to dryness. More preferred are the crystals of reduced coenzyme Q_{10} obtainable by crystallization or concentration to dryness following reduction of oxidized coenzyme Q_{10}. Particularly preferred are the crystals of reduced coenzyme Q_{10} obtainable by crystallization following reduction of oxidized coenzyme Q_{10}.

The solvent to be used in obtaining such crystals is not particularly restricted but includes hydrocarbons, fatty acid esters, ethers, alcohols, fatty acids, ketones, nitrogen-containing compounds (including nitriles and amides), sulfur-containing compounds, water, and the like.

As the hydrocarbons, fatty acid esters, ethers, alcohols, fatty acids, ketones, nitriles, amides, and sulfur-containing compounds, there may be mentioned such solvents as mentioned hereinabove.

As the nitrogen-containing compounds other than nitriles or amides, there may be mentioned, for example, nitromethane, triethylamine, pyridine, and the like.

For obtaining high-quality crystals of reduced coenzyme Q_{10} while suppressing the undesirable oxygen-involving side reaction, it is preferred to use a solvent with high protective effect against such oxidation, namely at least one species selected from among the above-mentioned hydrocarbons, fatty acid esters, ethers, and nitriles. Among them, hydrocarbons and fatty acid esters are more preferred as such solvent, hydrocarbons are still more preferred, and heptanes are particularly preferred.

The use of such an alcohol and/or ketone as mentioned hereinabove is also preferred because crystals of reduced coenzyme Q_{10} which have good slurry and crystal characteristics can be obtained when crystallization, which will be mentioned below as a method for recovering crystals, is utilized and such a solvent is used in the step of crystallization.

The crystallization of reduced coenzyme Q_{10} can be carried

out by the general crystallization procedure, for example cooling, concentration, solvent substitution, and use of a poor solvent, as used singly or in appropriate combination thereof. In particular, the cooling operation (crystallization by cooling) is preferably performed singly or in combination with some other operations.

For the crystallization of reduced coenzyme Q_{10}, it is very effective to purify and crystallize reduced coenzyme Q_{10} with simultaneous removal of impurities contained in the reaction mixture or extract obtainable in the conventional manner or produced by the above-mentioned reduction method or the like. This makes it possible to remove coexisting impurities, in particular analogous compounds having a similar structure and generally not always easy to remove (specifically, reduced coenzyme Q_9, reduced coenzyme Q_8, reduced coenzyme Q_7, etc). Alcohols and/or ketones are particularly effective solvents for removing the compounds having similar structures as mentioned above.

Furthermore, when an alcohol and/or a ketone are (is) used and a small amount of water is allowed to coexist, the solubility of reduced coenzyme Q_{10} can be properly reduced to give an increased yield. In addition, the slurry characteristics can be improved and, in particular, the solid-liquid separability (filterability) can be markedly improved, which is worthy of notice.

The mixing proportion between water and the alcohol and/or ketone solution may vary depending on the solvent species, hence cannot be absolutely specified. Any solvent substantially comprising the above-mentioned alcohol and/or ketone as a main component(s) can be used without any particular restriction. Namely, the proportion between the above alcohol and/or ketone in mixed solvent containing water has the lower limit of generally about 90 w/w%, preferably about 91 w/w%, more preferably about 92 w/w%, and still more preferably 93 w/w%, and the upper limit of about 99.5 w/w%, preferably about 99 w/w%,

more preferably about 98 w/w%, and still more preferably about 97 w/w%. In general, the crystallization can be favorably carried out at about 90 to about 99.5 w/w%, and most preferably at about 93 to about 97 w/w%.

The crystallization temperature of reduced coenzyme Q_{10} may vary depending on the crystallization solvent species and/or the method of crystallization, among others, hence cannot be absolutely specified. Generally, however, it is not higher than about 25°C, preferably not higher than about 20°C, more preferably not higher than about 15°C, and still more preferably not higher than about 10°C. The lower limit is the solidification temperature of the system. Generally, the crystallization is carried out at about 0 to about 25°C.

For minimizing immixture of various impurities into reduced coenzyme Q_{10} obtained or for obtaining slurry having good properties, the amount of precipitation of crystals per unit time can be controlled in the step of crystallization. A preferred rate of crystallization per unit time is, for example, not higher than the rate at which about 50% of the whole amount of crystals crystallizes out per unit time (50% amount/hour), and preferably not higher than the rate at which about 25% of the whole amount of crystals crystallizes out (25% amount/hour). The rate of cooling in cooling crystallization is generally not higher than about 40°C/hour, and preferably not higher than about 20°C/hour.

The crystallization of reduced coenzyme Q_{10} is preferably carried out under forced flowing. For inhibiting the occurrence of supersaturation and effecting the nucleation and crystal growth smoothly, or from the quality improvement viewpoint, the flowing is preferably caused by a power required for stirring per unit volume of generally not less than about 0.01 kW/m^3, preferably not less than about 0.1 kW/m^3, and more preferably not less than about 0.3 kW/m^3. The above forced flowing is generally provided by rotation of a stirring blade(s). If the above flowing is attained, however, it is not always

necessary to use a stirring blade(s). For example, the circulation of the liquid or the like procedure may be utilized.

For inhibiting the occurrence of supersaturation and effecting the nucleation and crystal growth smoothly in the step of crystallization, addition of seed crystals is preferred.

The crystallization concentration may vary depending on the crystallization solvent species and the method of crystallization, hence cannot be absolutely specified. For example, the weight of reduced coenzyme Q_{10} relative to the crystallization solvent weight at the time of completion of crystallization is not more than about 15 w/w%, preferably not more than about 13 w/w%, and more preferably not more than about 10 w/w%. From the productivity viewpoint, the lower limit is generally not lower than about 1 w/w%, preferably not lower than about 2 w/w%, and more preferably not lower than about 5 w/w%. The crystallization can be favorably carried out generally at about 5 to about 10 w/w%.

The thus-obtained crystals of reduced coenzyme Q_{10} can be recovered as a wet product, for example, by such a solid-liquid separation technique as centrifugation, pressure filtration, or vacuum filtration, if necessary followed by cake washing. Moreover, they can be recovered also as a dry product by further charging the wet product in a reduced pressure drier (vacuum drier) internally purged with an inert gas and drying the same under reduced pressure. The recovery in a dry form is preferred.

Next, the oil of reduced coenzyme Q_{10} to be used in the purification method of the present invention is now described. As described hereinabove, the oil of reduced coenzyme Q_{10} may be oil of reduced coenzyme Q_{10} obtainable by reducing an already existing oil of oxidized coenzyme Q_{10}, oil resulting from melting of crystals of reduced coenzyme Q_{10}, or oil obtainable by concentrating a reduced coenzyme Q_{10}-containing solution at a temperature not lower than the melting temperature. Needless to say, the oil obtainable by concentrating a reduced coenzyme

Q_{10}-containing solution, which is given by the above-mentioned reduction method, at a temperature not lower than melting temperature is also available.

The reduced coenzyme Q_{10}-containing organic phase to be used for obtaining oil of reduced coenzyme Q_{10} is not particularly restricted but, for inhibiting the undesirable oxygen-involving side reaction to thereby obtain a high-quality oil of reduced coenzyme Q_{10}, it is preferably a solution in a solvent with high protective effect against such oxidation, namely in at least one solvent selected from among hydrocarbons, fatty acid esters, ethers, and nitriles. Among them, hydrocarbons and fatty acid esters are more preferred as the solvent. Hydrocarbons are still more preferred, and heptanes are most preferred. The reduced coenzyme Q_{10}-containing organic phase may be the above-mentioned solution or a concentrate obtainable by concentrating the solution in the general manner.

In concentrating the reduced coenzyme Q_{10}-containing organic phase, the concentration of said organic phase is carried out at a temperature equal to or higher than the melting temperature of the concentrate comprising reduced coenzyme Q_{10} as a main component so that the coexisting solvent may be completely or nearly completely distilled off. As a result, an oily product of reduced coenzyme Q_{10}, from which solvents are completely or nearly completely removed, can be obtained. When the melting temperature is broad, the temperature is applicable as long as it is not lower than temperature at initiation of the melting.

In the practice of the invention, the temperature of concentration above for obtaining oil of reduced coenzyme Q_{10} may vary depending on the amount of the coexisting organic solvent, hence cannot be absolutely specified. It is, however, for example, preferably not lower than about 40°C, more preferably not lower than about 45°C, still more preferably not lower than about 48°C, particularly preferably not lower than

about 50°C, and most preferably about 60°C. The concentration can be favorably carried out generally within the range of about 40 to about 140°C, preferably about 40 to about 100°C, and more preferably about 50 to about 80°C, although the temperature depends on the solvent species and the amount thereof. The concentration is carried out at ordinary pressure or under reduced pressure.

The method mentioned above makes it possible to favorably obtain reduced coenzyme Q_{10} as an oily product by completely distilling off the organic solvent without any stirring trouble even when the purity of reduced coenzyme Q_{10} in the organic phase is, for example, not lower than about 80% by weight, preferably not lower than about 90% by weight, and more preferably not lower than 95% by weight.

When the oil of reduced coenzyme Q_{10} is obtained by distilling off the solvent, content of the solvent in the oil of reduced coenzyme Q_{10} is generally not higher than about 10% by weight, preferably not higher than about 5% by weight, and more preferably not higher than about 2% by weight, of the whole weight of the oil.

As described above, water-soluble impurities remaining in reduced coenzyme Q_{10}, in particular a reducing agent and/or impurities derived from the reducing agent, can be efficiently removed by the method of the present invention which is excellent in operationality.

The product reduced coenzyme Q_{10} obtainable by the purification method of the invention is of very high quality, and the weight of water-soluble impurities contained in the reduced coenzyme Q_{10} is expected to be 0.15% or lower, preferably 0.10% or lower, or more preferably 0.08% or lower.

BEST MODE FOR CARRYING OUT THE INVENTION

The following examples illustrate the present invention further in detail. These examples are, however, by no means limitative of the scope of the invention.

In the examples, the content of L-ascorbic acid was determined by HPLC, and the sodium hyposulfite and sodium hyposulfite-derived impurity content was determined by ion chromatography for determination of the sodium content, which was then converted into the sodium hyposulfite content. It is to be understood, however, that the reducing agent and/or reducing agent-derived impurity contents shown will never indicate the limit of purification of reduced coenzyme Q_{10} by the method of the invention.

(Production Example 1)

Oxidized coenzyme Q_{10} (100 g) and 60 g of L-ascorbic acid were added to 1000 g of ethanol, and the reduction reaction was carried out with stirring at 78°C. After 30 hours, the mixture was cooled to 50°C, and 400 g of ethanol and 100 g of water were added while maintaining the same temperature. This ethanol solution (containing 100 g of reduced coenzyme Q_{10}) was cooled to 2°C at a cooling rate of 10°C/hour with stirring (power for stirring: 0.3 kW/m^3), and thereby white slurry was obtained. The slurry obtained was filtered under reduced pressure, and the wet crystals were dried under reduced pressure (20 to 40°C, 1 to 30 mm Hg) to give 101 g of dry white crystals (containing 3.2% of L-ascorbic acid and 0.36% of oxalic acid). All the operations other than the drying under reduced pressure were performed in a nitrogen atmosphere.

(Example 1 and Comparative Example 1)

Ten-gram portions of the crystals of reduced coenzyme Q_{10} (containing 3.2% of L-ascorbic acid and 0.36% of oxalic acid) as obtained in Production Example 1 were respectively added to 190 g each of aqueous ethanol solutions differing in ethanol content to give slurries. Each slurry was stirred at 25°C for 10 minutes. This slurry was filtered under reduced pressure, and the wet crystals were dried under reduced pressure (20 to 40°C, 1 to 30 mm Hg) to give dry white crystals. The contents

of L-ascorbic acid and oxalic acid remaining in the thus-obtained crystals and the recovery percentage of reduced coenzyme Q_{10} are shown in Table 1. All the operations other than the drying under reduced pressure were performed in a nitrogen atmosphere.

As Comparative Example 1, the result, obtained when 190 g of water is added in lieu of 190 g of ethanol in above Example 1, is also shown. In the Comparative Example 1, the liquid properties were very poor, for example crystals adhered to the wall surface, and the discharge operation was very difficult to carry out.

Table 1

	Content of ethanol (%)	Content of L-ascorbic acid (%)	Content of oxalic acid (%)	Recovery percentage of reduced coenzyme Q_{10} (%)
Example 1	10	0. 07	0. 05	99
	30	0. 06	0. 03	99
	50	0. 06	0. 02	99
	90	0. 05	0. 04	97
Compar.Example 1	0	0. 18	0. 15	97

(Example 2)

A 10-gram portion of the crystals of reduced coenzyme Q_{10} (containing 3.2% of L-ascorbic acid and 0.36% of oxalic acid) as obtained in Production Example 1 was added to 190 g of ethanol to give slurry, which was stirred at 2°C for 10 minutes. This slurry was filtered under reduced pressure, and the wet crystals as dried under reduced pressure (20 to 40°C, 1 to 30 mm Hg) to give dry white crystals. All the operations other than the drying under reduced pressure were performed in a nitrogen atmosphere. In this case, the content of L-ascorbic acid remaining in the crystals was 0.06% and that of oxalic acid was 0.07%, and the recovery percentage of reduced coenzyme Q_{10} was 96%.

(Example 3 and Comparative Example 2)

A 10-gram portion of the crystals of reduced coenzyme Q_{10} (containing 3.2% of L-ascorbic acid and 0.36% of oxalic acid) as obtained in Production Example 1 was converted to an oily form at 60°C. Thereto was added 190 g of a 30% (by weight) aqueous ethanol solution, and the mixture was stirred at the same temperature for 10 minutes. Thereafter, the mixture was cooled to 25°C to convert the oil to crystals. The resulting slurry was filtered under reduced pressure, and the wet crystals were dried under reduced pressure (20 to 40°C, 1 to 30 mm Hg) to give dry white crystals. The contents of L-ascorbic acid and oxalic acid remaining in the thus-obtained crystals and the recovery percentage of reduced coenzyme Q_{10} are shown in Table 2. All the operations other than the drying under reduced pressure were performed in a nitrogen atmosphere.

As Comparative Example 2, the result, obtained when 190 g of water is added in lieu of 190 g of ethanol in above Example 3, is also shown. In the Comparative Example 2, the liquid properties were very poor, for example the oil of reduced coenzyme Q_{10} would not be uniformly dispersed and, even after cooling, crystals adhered to the stirring blade, and the discharge operation was very difficult to carry out.

Table 2

	Content of ethanol (%)	Content of L-ascorbic acid (%)	Content of oxalic acid (%)	Recovery percentage of reduced coenzyme Q_{10} (%)
Example 3	30	0.02	0.03	99
Compar. Example 2	0	0.43	0.19	97

(Example 4)

A 10-gram portion of the crystals of reduced coenzyme Q_{10} (containing 3.2% of L-ascorbic acid and 0.36% of oxalic acid) as obtained in Production Example 1 was added to 190 g of a 30% (by weight) acetone solution in water to give slurry, which was

stirred at 25°C for 10 minutes. This slurry was filtered under reduced pressure, and the wet crystals were dried under reduced pressure (20 to 40°C, 1 to 30 mm Hg) to give dry white crystals. All the operations other than the drying under reduced pressure were performed in a nitrogen atmosphere. In this case, the content of L-ascorbic acid remaining in the crystals was 0.09% and that of oxalic acid was 0.04%, and the recovery percentage of reduced coenzyme Q_{10} was 99%.

(Production Example 2)

Oxidized coenzyme Q_{10} (100 g) was dissolved in 1000 g of heptane at 25°C. Thereto was added gradually an aqueous solution, prepared as a reducing agent by adding 1000 ml of water to 100 g of sodium hyposulfite (purity at least 75%), for dissolution with stirring (power for stirring 0.3 kW/m^3), and the reduction reaction was carried out at 25°C and at pH 4 to 6. After 2 hours of the reaction, the mixture was cooled to 2°C at a cooling rate of 10°C/hour with continued stirring (power for stirring 0.3 kW/m^3), and thereby white slurry was obtained. All the above operations were carried out in a nitrogen atmosphere. The slurry obtained was filtered under reduced pressure, and the wet crystals were dried under reduced pressure (20 to 40°C, 1 to 30 mm Hg) to give 95 g of dry white crystals (containing 1.0% of sodium hyposulfite and sodium hyposulfite-derived impurities as expressed totally in terms of sodium hyposulfite). All the operations other than the drying under reduced pressure were performed in a nitrogen atmosphere.

(Example 5 and Comparative Example 3)

A 10-gram portion of the crystals of reduced coenzyme Q_{10} (containing 1.0% of sodium hyposulfite and sodium hyposulfite-derived impurities as expressed totally in terms of sodium hyposulfite) as obtained in Production Example 2 was purified by 1 hour of stirring in the same manner as in Example

1. The total amount of sodium hyposulfite and sodium hyposulfite-derived impurities remaining in the thus-obtained crystal and the recovery percentage of reduced coenzyme Q_{10} are shown in Table 3.

As Comparative Example 3, the result, obtained when 190 g of water is added in lieu of 190 g of ethanol in above Example 5, is also shown. In the Comparative Example 3, the liquid properties were very poor, for example crystals adhered to the wall surface, and the discharge operation was very difficult to carry out.

Table 3

	Content of ethanol (%)	The total amount of sodium hyposulfite and sodium hyposulfite-derived impurities remaining in reduced coenzyme Q_{10} (%)	Recovery percentage of reduced coenzyme Q_{10} (%)
Example 5	30	0.08	99
Compar. Example 3	0	0.18	97

(Example 6 and Comparative Example 4)

A 10-gram portion of the crystals of reduced coenzyme Q_{10} (containing 1.0% of sodium hyposulfite and sodium hyposulfite-derived impurities as expressed totally in terms of sodium hyposulfite) as obtained in Production Example 2 was stirred in a 30% (by weight) aqueous ethanol solution at 60°C for 1 hour. Thereafter, the aqueous phase was removed at the same temperature to give oil of reduced coenzyme Q_{10}. This oil was dropped onto a plate (40°C) with reduced coenzyme Q_{10} own crystals spread thereon to give a semispherical solid. The total amount of sodium hyposulfite and sodium hyposulfite-derived impurities remaining in the thus-obtained solid and the recovery percentage of reduced coenzyme Q_{10} are shown in Table 4.

As Comparative Example 4, the result, obtained when 190 g of water is added in lieu of 190 g of ethanol in above Example

5, is also shown. In the Comparative Example 4, the oil of reduced coenzyme Q_{10} would not be uniformly dispersed, and the liquid properties were thus very poor.

Table 4

	Content of ethanol (%)	The total amount of sodium hyposulfite and sodium hyposulfite-derived impurities remaining in reduced coenzyme Q_{10} (%)	Recovery percentage of reduced coenzyme Q_{10} (%)
Example 6	30	0.15	99
compar. Example 4	0	0.35	97

(Reference Example 1)

One-gram portions of reduced coenzyme Q_{10} (reduced coenzyme Q_{10}/oxidized coenzyme Q_{10} weight ratio 99.6/0.4) were respectively dissolved in 20 g each of various solvents specified in Table 5 at 25°C. After 24 hours of stirring in the air at 25°C, the reduced coenzyme Q_{10}/oxidized coenzyme Q_{10} weight ratio in each solution was determined. The results thus obtained are shown in Table 5.

Table 5

	R
Heptane	99.1/0.9
Hexane	98.7/1.3
Toluene	98.8/1.2
Chloroform	98.9/1.1
Ethyl acetate	98.9/1.1
Methyl tert-butyl ether	98.6/1.4
Tetrahydrofuran	98.5/1.5

R: Reduced coenzyme Q_{10}/Oxidized coenzyme Q_{10} weight ratio

(Reference Example 2)

One-gram portions of reduced coenzyme Q_{10} (reduced coenzyme Q_{10}/oxidized coenzyme Q_{10} weight ratio 99.6/0.4) were respectively dissolved in 100 g each of various solvents specified in Table 6 at 35°C. After 24 hours of stirring in the air at 35°C, the reduced coenzyme Q_{10}/oxidized coenzyme Q_{10} weight ratio in each solution was determined. The results thus obtained are shown in Table 6.

Table 6

Solvent	R
Heptane	96.7/3.3
Ethyl acetate	96.4/3.6
Acetonitrile	96.0/4.0

R: Reduced coenzyme Q_{10}/Oxidized coenzyme Q_{10} weight ratio

INDUSTRIAL APPLICABILITY

The invention, which has the constitution described hereinabove, makes it possible to conveniently and efficiently purify reduced coenzyme Q_{10} by a method with good operationality. The method of the present invention is suitable for an industrial scale production, and thereby high quality reduced coenzyme Q_{10} can be obtained.

CLAIMS

1. A method of purifying reduced coenzyme Q_{10} which comprises washing crystals and/or oil of reduced coenzyme Q_{10} with a water-soluble organic solvent or a mixed solvent composed of a water-soluble organic solvent and water to thereby remove a water-soluble impurity from the crystals and/or oil of reduced coenzyme Q_{10}.

2. The method of purifying reduced coenzyme Q_{10} according to Claim 1, wherein the washing of the crystals and/or oil of reduced coenzyme Q_{10} is carried out in a state of dispersion of the crystals and/or oil of reduced coenzyme Q_{10} in the water-soluble organic solvent or the mixed solvent composed of the water-soluble organic solvent and water.

3. The method of purifying reduced coenzyme Q_{10} according to Claim 2, wherein the dispersion is caused in a state of forced flowing.

4. The method of purifying reduced coenzyme Q_{10} according to any of Claims 1 to 3, wherein the water-soluble organic solvent comprises at least one species selected from among alcohols, ketones, ethers, and nitriles.

5. The method of purifying reduced coenzyme Q_{10} according to Claim 4, wherein the water-soluble organic solvent is ethanol.

6. The method of purifying reduced coenzyme Q_{10} according to any of Claims 1 to 5, wherein the washing is carried out with a mixed solvent

composed of an organic solvent and water.

7. The method of purifying reduced coenzyme Q_{10} according to Claim 6,

wherein the washing is carried out with a mixed solvent having a water-soluble organic solvent content of not less than 5 w/w%.

8. The method of purifying reduced coenzyme Q_{10} according to any of Claims 1 to 7,

wherein the water-soluble impurity is a reducing agent used for converting oxidized coenzyme Q_{10} into reduced coenzyme Q_{10} and/or an impurity derived from a reducing agent.

9. The method of purifying reduced coenzyme Q_{10} according to Claim 8,

wherein the reducing agent and/or the impurity derived from a reducing agent are/is hyposulfurous acid or a salt thereof and/or an impurity derived from hyposulfurous acid or a salt thereof.

10. The method of purifying reduced coenzyme Q_{10} according to Claim 8,

wherein the reducing agent and/or the impurity derived from a reducing agent are/is ascorbic acid or a related compound thereof and/or an impurity derived from ascorbic acid or a related compound thereof.

11. The method of purifying reduced coenzyme Q_{10} according to Claim 10,

wherein the impurity derived from ascorbic acid or a related compound thereof is oxalic acid.

12. The method of purifying reduced coenzyme Q_{10} according to any of Claims 1 to 11,

wherein the concentration of reduced coenzyme Q_{10} during washing is not higher than 30 w/w% as expressed in terms of the weight of reduced coenzyme Q_{10} relative to the weight of the solvent at the time of completion of the washing.

13. The method of purifying reduced coenzyme Q_{10} according to any of Claims 1 to 12,

wherein reduced coenzyme Q_{10} occurs as a form of crystals.

14. The method of purifying reduced coenzyme Q_{10} according to Claim 13,

wherein the washing temperature is not higher than 50°C.

15. The method of purifying reduced coenzyme Q_{10} according to any of Claims 1 to 14,

wherein reduced coenzyme Q_{10} occurs as a form of oil and the washing temperature is not lower than the melting temperature of reduced coenzyme Q_{10}.

16. The method of purifying reduced coenzyme Q_{10} according to Claim 15,

wherein the washing temperature is not lower than 40°C.

17. The method of purifying reduced coenzyme Q_{10} according to Claim 15 or 16,

wherein crystals of reduced coenzyme Q_{10} is recovered by cooling the solution obtainable after impurity removal from the oil of reduced coenzyme Q_{10}.

18. The method of purifying reduced coenzyme Q_{10} according to Claim 15 or 16,

wherein crystals of reduced coenzyme Q_{10} is recovered by contacting seed crystals to oil of reduced coenzyme Q_{10} obtainable after impurity removal from said oil.

19. The method of purifying reduced coenzyme Q_{10} according to any of Claims 1 to 18

wherein reduced coenzyme Q_{10} is purified in a deoxygenated atmosphere.

ABSTRACT

The object of the present invention is to provide a method of purifying reduced coenzyme Q_{10} to produce a high-quality product which is useful as an ingredient in foods, functional nutritive foods, specific health foods, nutritional supplements, nutrients, animal drugs, drinks, feeds, cosmetics, medicines, remedies, preventive drugs, etc., by a efficient manner suitable for an industrial scale production.

The present invention relates to a method of purifying reduced coenzyme Q_{10} which comprises washing crystals and/or oil of reduced coenzyme Q_{10} with a water-soluble organic solvent or a mixed solvent composed of a water-soluble organic solvent and water to thereby remove water-soluble impurities, especially a reducing agent or impurities derived from a reducing agent, from the crystals and/or oil of reduced coenzyme Q_{10}. The present invention makes it possible to conveniently and efficiently purify reduced coenzyme Q_{10} in a manner excellent in operationality, and to obtain a high-quality reduced coenzyme Q_{10}.